"INVITRO AND *INVIVO* EVALUATION OF ANTIMICROBIAL ACTIVITY OF AQUEOUS ALCOHOLIC EXTRACTS OF *ENICOSTEMMA LITTORALE* AND *LAGENARIA SICERARIA"*

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI- 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

Branch-IV- PHARMACOLOGY

Submitted by

REG.No.261625201

Under the Guidance of

Mr.V.VENKATESWARAN .M.Pharm.,

DEPARTMENT OF PHARMACOLOGY.

J.K.K. NATTARAJA COLLEGE OF PHARMACY

KUMARAPALAYAM – 638183

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CERTIFICATES

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"*Invitro* and *Invivo* Evaluation of Antimicrobial Activity of Aqueous Alcoholic Extracts Of *Enicostemma littorale* and *Lagenaria siceraria*".**Submitted by the student bearing **Reg.No: 261625201** to "**The Tamil Nadu Dr.M.G.R.Medical University – Chennai**", in partial fulfilment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on.....

Internal Examiner **External Examiner**

CERTIFICATE

This is certify that the work embodied in this dissertation entitled ***Invitro* and *invivo* evaluation of antimicrobial activity of aqueous alcoholic extract of *Enicostemma littorale* and *Lagenaria siceraria*.**submitted to "**The TamilNadu Dr.M.G.R.Medical University-Chennai**", Inpartial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmacology**,is a bonafide work carried out by the student bearing Reg.No.**261625201** during the academic year 2016-2018, under the guidance and supervision of **Mr.V.Venkateswaran,M.Pharm.,Professor, Dr.R.Sambath kumar, M.Pharm.,Ph.D,.Professor and Principal., Dr.R.Shanmugasundaram. M.Pharm., Ph.D., HOD and VicePrincipal** of J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

Dr.R.Sambathkumar, M. Pharm., PhD.,
Professor and Principal,
J.K.K. Nattraja College of Pharmacy.
Kumarapalayam- 638 183.

Dr.R Shanmugasundaram. M.Pharm.PhD.,
Vice Principal,&Head of the department,
Department of Pharmacology
J.K.K. Nattraja College of Pharmacy.
Kumarapalayam- 638 183.

Mr.V.Venkateswaran.,M.Pharm.,
Assistant professor,
Department of Pharmacology,
J.K.K.Nataraja college of pharmacy.
Kumarapalayam.638 183.

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled" ***"INVITRO* AND *INVIVO* EVALUATION OF ANTIMICROBIAL ACTIVITY OF AQUEOUS ALCOHOLIC EXTRACTS OF *ENICOSTEMMA LITTORALE* AND *LAGENARIA SICERARIA* ",**submitted to"**TheTamilNaduDr.M.G.R.Medical University-Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmacology** ,is a bonafide work carried out by the student bearing **Reg.No. 261625201** during the academic year 2016-2018, under my guidance and direct supervisionin the Department of Pharmacology, J.K.K.Nattraja College of Pharmacy,Kumarapalayam.

Place: Kumarapalayam

Date:

Mr.V.Venkateswaran, M. Pharm.,
Assistant Professor ,
Department of Pharmacology,
J.K.K. Nattraja College of Pharmacy.
Kumarapalayam- 638 183.

DECLARATON

"***INVITRO* AND *INVIVO* EVALUATION OF ANTIMICROBIAL ACTIVITY OF AQUEOUS ALCOHOLIC EXTRACT OF *ENICOSTEMMA LITTORALE* AND *LAGENARIA SICERARIA*"** submitted to "**The Tamil Nadu Dr.M.G.R Medical University - Chennai",** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work has been carried out by me during the academic year 2016-2018, under the guidance and supervision of **Mr. V.Venkateswaran., M.Pharm., Assistant Professor**, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma,associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place: Kumarapalayam **M.AMMU**

Date: **Reg.no261625201**

Dedicated to Parents, Teachers & My family

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M.AMMU

Reg.no.261625201

INTRODUCTION

1.1. Microorganism and pathology

The science dealing with the study of the prevention and treatment of diseases caused by micro-organisms is known as medical microbiology. Its sub disciplines are virology (study of viruses), bacteriology (study of bacteria), mycology (study of fungi), phycology (study of algae) and protozoology (study of protozoa). For the treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobial agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders. The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bacteriocides (killing bacteria), algicides (killing algae) or fungicides (killing fungi).

Infection is an important cause of morbidity and mortality in hospitalized burn patients. In spite of considerable advances in medicine and specific treatment of burn, infection continues to pose the greatest danger to burn patients and approximately 73% of all deaths within the first 5 days post burn are directly or indirectly caused by septic processes. The rate of nosocomial infections is higher in burn patients due to various factors like nature of burn injury itself, immune compromised status of the patient, age of the patient, extent of injury, and depth of burn in combination with microbial factors such as type and number of organisms, enzyme and toxin production, colonization of the burn wound site, systemic dissemination of the colonizing organisms. In addition, cross-infection results between different burn patients due to overcrowding in burn wards. The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin; thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients. Currently the common pathogens isolated from burn patients are Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella spp. and various coliform bacilli. Multidrug-resistant bacteria have frequently been reported as the

cause of nosocomial outbreaks of infection in burn units or as wound colonizers in burn patients

1.2. Classification of Antibacterial Agents

The antibacterial agents are classified in three categories:

Antibiotics and chemically synthesized chemotherapeutic agents. (II) Non-antibiotic chemotherapeutic agents (Disinfectants, antiseptics and preservatives) (III) Immunological products.

Antibiotics they are produced by micro-organisms or they might be fully or partly prepared by chemical synthesis. They inhibit the growth of micro-organisms in minimal concentrations. Antibiotics may be of microbial origin or purely synthetic or semisynthetic.

They can be classified by manner of biosynthesis or chemical structure.

Synthetic antimicrobial agents include sulfonamides, diamino pyrimidine derivatives, antitubercular compounds, nitrofuran compounds, 4-quinoline antibacterials, imidazole derivatives, flucytosine etc. (II) Non-antibiotics The second category of antibacterial agents includes non-antibiotic chemotherapeutic agents which are as follows:

1.2.1. Acids and their derivatives

Some organic acids such as sorbic, benzoic, lactic and propionic acids are used for preserving food and pharmaceuticals. Salicyclic acid has strong antiseptic and germicidal properties as it is a carboxylated phenol. The presence of –COOH group appears to enhance the antiseptic property and to decrease the destructive effect. Benzoic acid is used externally as an antiseptic and is employed in lotion and ointment. Benzoic acid and salicylic acid are used to control fungi that cause disease such as athlete’s foot. Benzoic acid and sodium benzoate are used as antifungal preservatives. Mandolic acid possesses good bacteriostatic and bactericidal properties.

1.2.2. Alcohols and related compounds

They are bactericidal and fungicidal, but are not effective against endospores and some viruses. Various alcohols and their derivatives have been used as antiseptics e.g. ethanol and propanol. The antibacterial value of straight chain alcohols increases with an increase in the molecular weight and beyond C8- the activity begins to fall off. The isomeric alcohol shows a drop in activity from primary, secondary to tertiary. Ethanol has extremely numerous uses in pharmacy.

Chlorination and compound containing chlorine Chlorination is extensively used to disinfect drinking water, swimming pools and for the treatment of effluent from industries. Robert Koch in 1981 first referred to the bactericidal properties of hypochlorites. N-chloro compounds are represented by amides, imides and amidines wherein one or more hydrogen atoms are replaced by chlorine.

1.2.3. Iodine containing compounds

Iodine containing compounds are widely used as antiseptic, fungicide and amoebicide. Iodophores are used as disinfectants and antiseptics. The soaps used for surgical scrubs often contain iodophores.

1.2.4. Heavy metals

Heavy metals such as silver, copper, mercury and zinc have antimicrobial properties and are used in disinfectant and antiseptic formulations. Mercurochrome and merthiolate are applied to skin after minor wounds. Zinc is used in antifungal antiseptics. Copper sulfate is used as algicides.

1.2.5. Oxidising agents

Their value as antiseptics depends on the liberation of oxygen and all are organic compounds.

1.2.6. Dyes

Organic dyes have been extensively used as antibacterial agents. Their medical significance was first recognized by Churchman3 in 1912. He reported inhibitory effect of Crystal violet on Gram-positive organism. The acridines exert

bactericidal and bacteriostatic action against both Gram-positive and Gramnegative organisms.

1.2.7. 8-Hydroxyquinolines.

Surface active agents Soaps and detergents are used to remove microbes mechanically from the skin surface. Anionic detergents remove microbes mechanically; cationic detergents have antimicrobial activities and can be used as disinfectants and antiseptics.

Immunological products certain immunological products such as vaccines and monoclonal antibodies are used to control the diseases as a prophylactic measure.

1.2.8. Mode of Action

Antimicrobial drugs interfere chemically with the synthesis of function of vital components of micro organisms. The cellular structure and functions of eukaryotic cells of the human body. These differences provide us with selective toxicity of chemotherapeutic agents against bacteria. Antimicrobial drugs may either kill microorganisms outright or simply prevent their growth. There are various ways in which these agents exhibit their antimicrobial activity.

They may inhibit

- Cell-wall synthesis
- Protein synthesis
- Nucleic acid synthesis
- Enzymatic activity
- Folate metabolism or
- Damage cytoplasmic membrane

Bacteriostatic dyes Stearns and Stearn6 attributed the bacteriostatic activity to triphenylmethane dyes. Fischer and Munzo7 have found the relationship between their structure and effectiveness of such dyes. A number of drugs are metal-binding agents. The chelates are the active form of drugs. The site of action within the cell or on the cell surface has not been established. The site of action of oxine and its analogs has been suggested inside the bacterial cell8 or on cell surface.9 Detoxification of

antibacterials P-Aminobenzoic acid is a growth factor for certain micro-organisms and competitively inhibits the bacteriostatic action of sulfonamides. The metabolites identified in man are p-amino-benzoylglucoronide; p-aminohippuric acid, p-acetylaminobenzoic acid. 8-Hydroxyquinoline (oxine) and 4-hydroxyquinoline are excerted as sulfate esters or glucorinides. Bacteria The bacteria are microscopic organisms with relatively simple and primitive forms of prokaryotic type. Danish Physician Christian Grams, discovered the differential staining technique known as Gram staining, which differentiates the bacteria into two groups “Gram positive” and “Gram negative”, Gram positive bacteria retain the crystal violet and resist decolorization with acetone or alcohol and hence appear deep violet in colour; while Gram negative bacteria, which loose the crystal violet, are counter-stained by saffranin and hence appear red in colour.

1.3. Antimicrobial Agents

Table No: 1: Classification of Antimicrobial agents

GENERIC NAME	TRADE NAMES	ROUTE
Antituberculous Agents		
Capreomycin	Capastat	i.m.
Cycloserine	Seromycin	Oral
Ethambutol	Myambutol	Oral
Ethionamide	Tecator-SC	Oral
Isoniazid	INH, Nydrazid	Oral, i.m.
Para-aminosalicylic acid		Oral
Pyrazinamide		Oral
Rifampin	Rifadin, Rimactane	Oral, i.v.
Streptomycin		i.m.
Antifungal Agents		
Amphotericin B	Fungizone	i.v.
Miconazole	Monistat	i.v.
Ketoconazole	Nizoral	Oral
Fluconazole	Diflucan	Oral, i.v.
Itraconazole	Sporanox	Oral
Flucytosine	Ancobon	Oral, i.v.
Antiviral Agents		
Acyclovir	Zovirax	Oral, i.v.
Ganciclovir	Cytovene	i.v.
Amantadine	Symmetrel	Oral
Rimantidine	Flumadine	Oral
Antibacterial Agents		
Aminoglycosides		
Amikacin	Amikin	i.m., i.v.
Gentamicin	Garamycin	i.m., i.v.
Tobramycin	Nebcin	i.m., i.v.
Netilmicin	Netromycin	i.m., i.v.

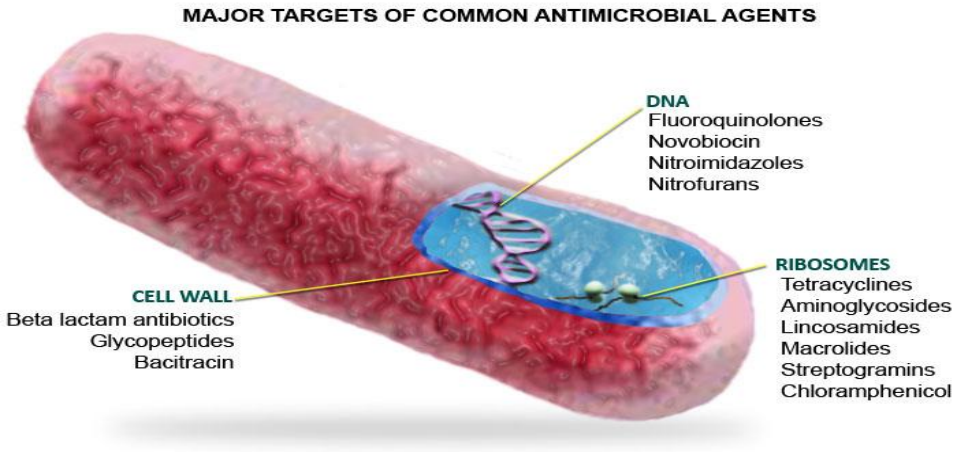
Cephalosporins		
1st Generation		
Cefadroxil	Duricef	Oral
Cefazolin	Ancef, Kefzol	i.m., i.v.
Cephalexin	Keflex	Oral
2nd Generation		
Cefaclor	Ceclor	Oral
Cefamandole	Mandol	i.m., i.v.
Cefmetazole	Zefasone	i.v.
Cefonicid	Monocid	i.m., i.v.
Cefotetan	Cefotan	i.m., i.v.
Cefoxitin	Mefoxin	i.m., i.v.
Cefprozil	Cefzil	Oral
Loracarbef	Lorabid	Oral
Cefuroxime	Zinacef	i.m., i.v.
	Ceftin	Oral
3rd Generation		
Cefepime	Axepim	i.v.
Cefixime	Suprax	Oral
Cefoperazone	Cefobid	i.m., i.v.
Cefotaxime	Claforan	i.m., i.v.
Cefpodoxime	Vantin	Oral
Ceftazidime	Fortaz, Tazidime, Tazicef	i.m., i.v.
Ceftizoxime	Cefizox	i.v.
Ceftriaxone	Rocephin	i.m., i.v.
Penicillins		
Penicillin G potassium	Pfizerpen	i.m., i.v.
Penicillin G tablets		Oral
Penicillin V	Pen-Vee K	Oral
Ampicillin	Omnipen	i.m., i.v., Oral
Ampicillin/Sulbactam	Unasyn	i.v.

Amoxicillin	Amoxil	Oral
Amoxicillin/Clavulanate	Augmentin	Oral
Oxacillin	Bactocil	Oral
Nafcillin	Unipen	i.m., i.v., Oral
Cloxacillin	Tegopen	Oral
Dicloxacillin	Pathocil	Oral
Ticarcillin	Ticar	i.m., i.v.
Ticarcillin/Clavulanate	Timentin	i.v.
Mezlocillin	Mezlin	i.m., i.v.
Piperacillin	Pipracil	i.m., i.v.
Piperacillin/Tazobactam	Zosyn	i.v.
Other Beta-lactams		
Aztreonam	Azactam	i.m., i.v.
Imipenem-cilastatin	Primaxin	i.v.
Tetracyclines		
Tetracycline HCI	Achromycin	Oral, i.v.
Doxycycline	Vibramycin	Oral, i.v.
Trimethoprim-Sulfonamides		
Sulfisoxazole	Gantrisin	Oral, i.v.
Trimethoprim/Sulfamethoxazole	Septra, Bactrim	Oral, i.v.
Trimethoprim	Proloprim	Oral
Macrolides		
Erythromycin	many	Oral, i.v.
Clarithromycin	Biaxin	Oral
Azithromycin	Zithromax	Oral
Quinolones		
Norfloxacin	Noroxin	Oral
Ciprofloxacin	Cipro	Oral, i.v.
Ofloxacin	Floxin	Oral, i.v.
Enoxacin	Penetrex	Oral
Lomefloxacin	Maxaquin	Oral
Others		
Clindamycin	Cleocin	Oral, i.v.
Chloramphenicol	Chloromycetin	Oral, i.v.
Vancomycin	Vancocin, Vancoled	i.v.

1.4.1. General Mechanism of action of Antimicrobial agents

Different antibiotics have different modes of action, owing to the nature of their structure and degree of affinity to certain target sites within bacterial cells.

Figure No: 1: General Mechanism of action of Antimicrobials



1.4.2. Inhibitors of cell wall synthesis

While the cells of humans and animals do not have cell walls, this structure is critical for the life and survival of bacterial species. A drug that targets cell walls can therefore selectively kill or inhibit bacterial organisms. Examples: penicllins, cephalosporins, bacitracin and vancomycin.

1.4.3. Inhibitors of cell membrane function

Cell membranes are important barriers that segregate and regulate the intra- and extracellular flow of substances. A disruption or damage to this structure could result in leakage of important solutes essential for the cell's survival. Because this structure is found in both eukaryotic and prokaryotic cells, the action of this class of antibiotic are often poorly selective and can often be toxic for systemic use in the mammalian host. Most clinical usage is therefore limited to topical applications. Examples: polymixin B and colistin.

1.4.4. Inhibitors of protein synthesis

Enzymes and cellular structures are primarily made of proteins. Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cells. Several types of antibacterial agents target bacterial protein synthesis by binding to either the 30S or 50S subunits of the intracellular ribosomes. This activity then results in the disruption of the normal cellular metabolism of the bacteria, and consequently leads to the death of the organism or the inhibition of its growth and multiplication. Examples: Aminoglycosides, macrolides, lincosamides, streptogramins, chloramphenicol, tetracyclines.

1.4.5. Inhibitors of nucleic acid synthesis

DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in the process of DNA or RNA synthesis, which causes interference of the normal cellular processes which will ultimately compromise bacterial multiplication and survival. Examples: quinolones, metronidazole, and rifampin.

1.4.6. Inhibitors of other metabolic processes

Other antibiotics act on selected cellular processes essential for the survival of the bacterial pathogens. For example, both sulfonamides and trimethoprim disrupt the folic acid pathway, which is a necessary step for bacteria to produce precursors important for DNA synthesis. Sulfonamides target and bind to dihydropteroate synthase, trimethophrim inhibit dihydrofolate reductase; both of these enzymes are essential for the production of folic acid, a vitamin synthesized by bacteria, but not humans.

Alternative medicines have been practiced for centuries and remained as integral part of many civilizations around the globe. One important aspect of alternative medicine includes herbal medicines/drugs in which locally available plants or its parts are used in treating ailments. Herbal medicines are commonly used for treating both infectious and non-infectious diseases. On the other hand, Antimicrobials used to treat bacterial infections caused by multiple drug resistant (MDR) and total drug resistant (TDR) strains are becoming more common in the

clinical setting and world is looking for alternative therapies to treat such infections. Herbal medicines are anticipated to protect us from infections as they are considered as better alternatives for existing and emerging antimicrobial drug resistant (ADR) pathogens. Herbal antimicrobials acts either by killing or restricting the bacterial growth through parallel mechanisms as antibiotics similarly there could be mechanisms of herbal drug resistance just like antibiotic resistance in microbes. However, lack of systematic and standard data on herbal antimicrobial activity neither we could understand the extent of herbal drug resistance nor the mechanism of resistance in microbes. The recent studies on antimicrobial properties of herbal drugs on clinical isolates indicated that there is some insensitivity or resistance in microbes towards some common herbal antimicrobial compounds. This review focuses on recent reports of herbal drug resistance among pathogenic microbes (clinical bacterial isolates) against herbal drugs.

Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns in 21st century. World health organization's 2014 report on global surveillance of antimicrobial resistance states that "antibiotic resistance is a serious threat and no longer a prediction for the future; it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country". This jeopardizes the treatment of common infections in the community and hospital. It has also been predicted by several authors that the next pandemic will not be of some specific disease but due to ineffectiveness of available drugs to cure even small cuts and wounds.

Antimicrobial drug resistance (ADR) hampers the control of infectious diseases and has potential to threaten health security, damage trade and economies but it is difficult to think of "the world without antibiotics". It may be a deadly situation because the routine surgery, cancer treatments, organ transplants etc. become just impossible without antibiotics. So, we need to save antibiotics for certain therapeutic interventions. It is also important to take urgent and coordinated action to save the world from entering a post-antibiotic era, in which common infections and minor injuries can become life threatening.

Development of ADR is a natural phenomenon [2]. However, certain human actions accelerate the emergence and spread of ADR. Inappropriate therapeutic use of antimicrobial drugs, and use in agriculture, fish, poultry and animal farming, favours the emergence and selection of resistant strains. Besides, poor infection prevention & control practices further contribute for emergence and spread of ADR. Eminent organizations like WHO, World Organization for Animal Health (OIE) and Food and Agriculture Organization (FAO) of the United Nations have collaborated to promote best practices to avoid the emergence and spread of antibacterial resistance. All attempts are in progress to promote optimal use of antibiotics both in humans and animals to address problem of growing AMR.

Most of the pathogenic bacteria have developed resistance to modern antibiotics as a result of which we are evidencing multi drug resistance among bacteria. We are running out of antibiotics and could not add any new group of antibiotics since last three decades. At the same time, there is no potential antibiotic in pipeline for release in near future. As a result, research in alternative medicine has begun and one such alternative is use of herbal drugs to treat infections.

Since ancient times, herbs and their essential oils are known for their varying degrees of antimicrobial activity. Due to immense biodiversity, India is a vast repository of medicinal plants that are used in traditional medical treatments. Almost, 70% modern medicines in India are derived from natural products and the various indigenous systems of India such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. Herbal medicine has always been a part of Indian culture and gaining popularity due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures in India. It's reported that more than 500 Indian traditional communities use about 800 plant species for curing different diseases among 20,000 medicinal plant species that are available in the country.

The burning question is now, if non-judicious use of antibiotics may lead to emergence and spread of ADR why it may not happen to herbal antimicrobials. In recent past, a few reports have already documented about prevalence of herbal antimicrobial drug resistance (HADR) in environmental and clinical strains of bacteria. However, either we fear to accept the existence of HADR (probably due

to looking those as last resources) or due to poor understanding of HADR. Therefore, this review details about HADR from clinical bacterial isolates documented recently.

1.5. Drug Resistance in Bacteria

Emergence of drug resistance made treatment of infectious diseases more difficult. For instance, extensively drug-resistant tuberculosis (XDRTB) has been identified in 92 countries and there were about 4, 50,000 new cases of multidrug-resistant tuberculosis (MDR-TB) worldwide in the year 2012. Similarly, evolution of methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Staphylococcus* aureus (VRSA) leads to nosocomial infections. It is alarming fact that fluoroquinolone and carbapenem resistance in E. coli and other commensal intestinal bacteria are on continuous rise.

Antibiotic resistance is a phenomenon in which some subpopulations of bacteria resist the presence of one or more antibiotics and pathogens that are resistant to multiple antibiotics are considered as multidrug resistant (MDR) or superbugs. The evolution of resistant bacterial strains is a natural phenomenon which occurs when microorganisms replicate themselves erroneously or when resistant traits are exchanged between strains through horizontal gene transfer mechanisms. The use and misuse of antimicrobial drugs accelerates the emergence of drug-resistant strains. Poor infection control practices, inadequate sanitary conditions and inappropriate food-handling encourage further spread of the antimicrobial resistance. Moreover, the scenario of AMR is not only restricted to human pathogens, but also common in veterinary pathogens. It has been reported that extended spectrum β-lactamase and metallo-β-lactamase producing strains are common in animals and also present in their environment.

1.6. Need for Revival of Herbal Antimicrobials

Herbal medicines are derived from the plants or plant extracts containing therapeutic substances. The herbal medicine practice is generally called as complementary and alternative medicine (CAM). Many essential oils are relatively easy to obtain, have low mammalian cell toxicity, and degrade quickly in water and soil, making them relatively easy to use and environment friendly antibiotic alternatives.Herbal drugs are used by physicians for hundreds of years as indigenous systems of medicine and about 80% of the world population still use them for primary

health care. Hippocrates (5th century B.C.) in his writings mentioned approximately 300 to 400 medicinal plants. Similarly, Dioscorides (1st century B.C.) wrote De Materia Medica, a medicinal plant treatise that outlined the medical use of numerous plant species. China has history of 5000 years in use of herbal medicines. The Holy Bible also describes many medicinal plant species, such as myrrh and frankincense, which were reported to have antiseptic and healing properties. Around 250,000-500,000 plants species are found worldwide. Many of these plants are used for various purposes such as foods and medicines by both humans and animal species but less than 10% of these plants have been scientifically investigated. Herbal medicine is becoming more popular not only in developing countries but also in developed countries. Many studies have been conducted across the globe to prove or find the antimicrobial efficiency and or properties of herbal drugs. For example, *Achille millifolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa offficinalis* (lemon-balm), *Ocimum basilucum* (basil), *Psidium guajava* , (guava),*Punica granatum* (pomegranate), *Rosmarinusofficinalis* (rosemary), *Salvia officinali* (sage), *Syzygyum joabolanum* (jambolan), *Thymus vulgaris* (thyme) and phytochemicals such as benzoic acid, carvacrol, cinnamic acid, eugenol and farnesol were found to contain antimicrobial properties . Among herbal preparations, essential oils of several medicinal plants are often shown to possess antimicrobial activities. Among all the oils, the essential oil of cinnamon has been found to be the most effective, followed by the essential oil of oregano and thyme (the active ingredient in latter two plants is carvacrol.

The demand for the herbal drugs has increased in recent times, as many plants or herbs are scientifically proven to contain bioactive compound(s) and as alternatives to harmful synthetic drugs that cause side effects to biological system and environment. The herbal drugs have been used for treatment of many infectious diseases in humans as well as in animals all over world. In developing countries herbal medicines are now in great demand since they are not only inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Other than antimicrobial therapy, herbal drugs are used for treatment of age-related disorders like memory loss, osteoporosis, immune disorders, etc. The active ingredients of plants can also be used in laxatives, blood thinners, antibiotics and anti-malarial medications. Medicinal plants can also be used as sources of lead compounds for drug design and development. It has been reported that volatile

oils from plants have analgesic, antibacterial, deodorizing, febrifuge, fungicidal, antiseptic, antidepressant, astringent, diuretic, galactogogue, insecticidal, antipyretic, antimicrobial and sedative properties.

It is reported that the curcumin from turmeric inhibited the biofilm formation in *H. pylori* in cell cultures. However, *H. pylori* could restore ability to form biofilm during extended time of incubation. Some essential oils have been reported to kill biofilms formed by *Pseudomonas aeruginosa* (PAO1), *Pseudomonas putida*, and *Staphylococcus aureus*. There are innumerable uses of herbal medicines; therefore, there is a need to revamp research to develop alternative antimicrobial drugs for the treatment of infectious diseases. Of the several approaches, one is to screen local medicinal plants for possible antimicrobial properties and active molecules are important for the future. Another approach may be to find out the herbal molecules which potentiate the existing antibiotics through synergistic action or through inhibiting efflux pumps or inactivating antibiotic degrading enzymes of microbes.

1.7. Mechanism of Action of Herbal Drugs

Broadly, six possible mechanisms of antimicrobial action has been reported, they are: (1) disintegration of cytoplasmic membrane, (2) interaction with membrane proteins (ATPases and others), (3) disturbance of outer membrane of gram negative bacteria with the release of lipopolysaccharides, (4) destabilization of the proton motive force with leakage of ions, (5) coagulation of the cell content, and (6) inhibition of enzyme synthesis. The effects of essential oils and their bioactive constituents mainly disrupt the bacterial cell membranes followed by release of membrane components. However, it has been reported that the components of lemongrass oil also inhibited biofilm formation, killed preformed biofilms and have multiple targets on the bacterial cell. The lipophilic monoterpenes of essential oils deeply interact and affect the molecular structure of lipid bilayers. Some examples are myrtle essential oil which affects mainly cell wall and membrane structures leading to the release of intracellular contents accompanied by disruption of membrane function such as electron transfer, enzyme activity or nutrient absorption. Carvacrol and pcymene get absorbed by lipid membranes thus affecting membrane lipid composition. The antimicrobial activity of terpenes such as thymol also damages lipid

membranes. Cranberry has also been reported to adhere to uroepithelial cells and change the physicochemical surface properties of uropathogenic *E. coli.*

1.8. Herbal Drug Resistance

The resistance to herbal drugs in various clinical and or nonclinical isolates of pathogenic organisms has been reported more recently from veterinary clinical isolates but this resistance or sensitivity is comparative and results vary with the concentration of drug used. For example, studies on resistance to LGO and other herbal drugs showed varying degree of MIC depending upon species of microbes tested or within same species among different strains, this suggests that microbes has mechanism to overcome the bactericidal concentration of herbal drugs also.

The ability of microorganisms to develop resistance to herbal drugs is not well studied. It is often stated that bacteria can not develop resistance to herbal medicines. However, recent reports suggested that microbes can overcome bactericidal or bacteriostatic activities of the herbal drugs. Many of herbal drugs reported to contain better antimicrobial properties either alone or in combination with antibiotics but reports on ineffective herbal drugs on certain strains cannot be neglected. Many clinical and nonclinical bacterial isolates were sensitive to herbal drugs like *A. nilotica, T. arjuna, S. aromaticum* but study also revealed that some of the isolates such as *P. aeruginosa, E. coli, C. albicans, K. pneumoniae, E. coli* from nosocomial infections, and *E. coli, C. albicans, K. pneumoniae* isolated from community acquired infections were resistant to herbal drugs. Similarly, many bacterial strains isolated from different clinical conditions in animals and from post mortem cases were resistant to lemon grass oil. They also reported resistance in *E. coli* ATCC 25922, and field isolates of *E. coli, P. aeruginosa* and *S. flexneri* against aqueous extracts of unripe banana (*Musa sapientum*), lemon grass (*Cymbopogon citratus*) and turmeric (*Curcuma longa*). Lemon grass oil was reported to be effective against multi drug resistant bacteria except *P. aeruginosa*. *Moore-Neibel et al. 2011*, reported that the antimicrobial activity of lemongrass oil against Salmonella Newport was concentration and time dependent. Among bacterial population, the enteric bacteria are often reported to be more resistant than other bacteria to herbal drugs. High resistance was reported among bacterial strains of gecko origin to herbal antimicrobials, moreover, it is said that, herbal drug resistance varied among strains of

bacteria and herbal drugs can be selective and may not have broad spectrum against large bacterial populations.

One important use of herbal medicines is to treat the multiple drug resistant pathogens but, some drug resistant or MDR strains have also been isolated from the herbal products. Brown and Jiang could isolate ceftriaxone and tetracycline resistant bacteria from ground garlic samples at 1.1×10^2 CFU/g and 3.0×10^2 CFU/g, respectively. Similarly tetracycline-resistant bacteria were present in organic onion powder samples. The low levels of resistant bacteria were also isolated from other products such as ginger, rosemary, mustard, and goldenseal. High CFU count of enteric bacteria was found even in dry spice samples. The presence of drug resistant bacteria in the herbal medicinal products can also become a source of antibiotic resistance to commensel bacteria in consumers . Ogunshe and coworkers confirmed that most indigenous orally consumed herbal medications in Nigeria harbor bacterial flora that exhibited multiple resistance to routinely used antibiotics. Besides being source of MDR strains, resistance to herbal drugs is not uncommon among bacteria.

Most herbal products on the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness. Though the guidelines for the assessment of herbal medicine are developed by WHO, but it has not systematically evaluated . This may lead to indiscriminate or over use of these drugs which could cause herbal drug resistance. The ability of microorganisms to develop resistance to herbal drugs is not well studied. However recent reports suggested that microbes can overcome bactericidal or bacteriostatic activities of the herbal drugs, gives some examples of resistant and sensitive bacteria against common herbal agents reported in recent studies.

Table No: 3: Few herbs having antimicrobial agents

No	Herbal drug/ Essential oils tested	Effective on	In effective against
1	Lemon grass oil *(Cymbopogon Spp)* (Important constituents- citralgeraniol, myrcene,neral, limonene, piperitone, citronellal)	*S. aureus, B. cereus, B. subtilis, E. coli, K. pneumoniae*	*P. aeruginosa*
		Majority of *Bacillus* spp., *Streptoccocus* spp., *Aeromonas* spp., *Edwardsiella, Budvicia aquatic* and *Leminorellaghirmontii*	*Majority of Staphyloccous* spp., *Enterococcus* spp., *Salmonella enterica, Citrobacter* spp., *Providencia spp, Kluyveracryocrescens, Enterobacter* spp., *Proteus* spp., *Escherichia* spp., *Serratia* spp., *Erwiniaananas, Pragiafontium, Klebsiella* spp.
2	Sage essential oil (*Saviaofficinalis* L.) (Important constituents: pinene, camphene,myrcene, limonene, 1,8-cineole, thujone,camphor, linalool, bornyl acetate and borneol)	*Dermatophilus congolesis, Pasteurellacanis, Plesiomonasshigelloides and Streptococcus* spp.	*Staphylococcus aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Salmonella enterica* ssp. *Enterica*serovar Typhimurium
3	*Artemesia vulgaris* (Important constituents: Germacrene, Caryophyllene, Zingiberene,Borneol)	Yeast, mold and *Bacillus* strains	*Staphylococcus aureus*, *Streptococcus* spp., *E. coli, Salmonella* and *Klebsiella pneumonia A.hydrophila, E. tarda* strains.
4	Caraway essential oil *(Carumcarvi* L.) (Important composition: Acetaldehyde, Cumuninic aldehyde, Furfurol, Carvone, Limonene)	*Bacillus cereus, Bordetellabronchiseptica, Brucellaabortus, Dermatophilus congolensis, Erwiniaananas, Escherichia coli* (two),*Moraxella canis*(two),*Moraxella osloensis, Pasteurellamultocida, Proteus penneri, Pseudomonas*	Aeromonads

		aeruginosa, Raoultellaterrigena and *Streptococcus pyogenes.*	
5	Nutmeg essential oil *Myristica fragrans* (Important composition: Sabinene, Camphene, d-Pinene, Dipentene, d-Linalool, d-Borneol, i-Terpineol, Geraniol, Myristcin)	*Shigelladysenteriae, Proteus mirabilis, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa*	*Klebsiella pneumoniae, Salmonella typhi, Bacillus subtilis, Proteus vulgaris*
6	*Selinum wallichianum* Essential oil (or) Milk Parsley (Important composition: α-bisabolol, farnesol,germacrene D, citronellylpropanoate, α-bisabolol oxide B, sabinene, β-farnesene, limonene)	Reference strains of *E. coli*, (E3376 and E3382), *Edwar siellatarda*	*Bacillus coaggulans, E. coli, Aeromonas hydrophila, Lactobacillus acidophilus, Klebsiella pneumoniae, Enterococcus*
7	*Pelargonium*species Rose geranium oil (Important composition: a-pinene, myrcene, limonene, menthone, linalool, geranyl acetate, citronellol, geraniol and geranyl butyrate)	*Streptococcus equi* ssp. *equi, S. pyogenes, S. pneumoniae*	*Streptococcus equi* ssp. *zooepidemicus Staphyloccoccus* spp.
8	*Myrtuscommunis* L. (Important constituents: Myrtenyl acetate, 1,8-cineol, α-pinene, linalool, limonene, linalyl acetate, geranyl acetate, and α-terpineol)	*S. aureus, Micrococcus luteus, Streptococcus pneumoniae, S. pyogenes, S. agalactiae, Listeria monocytogenes, E. coli, Proteus vulgaris, Pseudomonas aeruginosa*	*Campylobacter jejuni*
9	*Acacia nilotica* (Important constituents: Menthol, limonene,Pinene)	*Streptococcus mutans* ATCC-700610, *S. bovis* ATCC 9809, *Staphylococcus aureus* ATCC-29213, *Enterococcus faecalis* ATCC-	*E.coli*(isolates of community acquired infection)

		29212, *Pseudomonas aeruginosa* ATCC-27853, *Salmonella* TphimuriumATCC-13311, *E. coli* ATCC-25922, *C. albicans* ATCC-10231, *K. pneumonia* ATCC-700603, *E. coli* (isolate of nosocomial infection), *E. coli*(isolates of community acquired infection), *C. albicans*(isolates of community acquired infection), *K.pneumonia* (isolates of community acquired infection)	
10	*Terminalia arjuna (Important constituents: Tannins, triterpenoidsaponins (arjunic acid, arjunolic acid, arjungenin and arjunic acid), flavonoids, gallic acid, ellagic acid, phytosterols.)*	*Streptococcus mutans ATCC-700610, S. bovis ATCC- 9809,S. aureus ATCC-29213, E. faecalis ATCC-29212.*	*P. aeruginosa ATCC-27853, S. Typhimurium ATCC-13311, E. coli ATCC-25922, C. albicans ATCC-10231, K. pneumonia ATCC-700603, E. coli(isolate of nosocomial infection), E. coli(isolates of community acquired infection), C. albicans(isolates of community acquired infection), K. pneumonia (isolates of community acquired infection)*
11	*Eucalyptus globules (Important constituents: α-pinene, 1,8-cineol, pinocarveol-trans)*	*S. mutans ATCC-700610, S. aureus ATCC-29213, E. faecalis ATCC-29212, S. bovis ATCC 9809.*	*27853, S. Typhimurium ATCC-13311, E. coli ATCC-25922, C. albicans ATCC-10231, K. pneumonia ATCC-700603, E. coli(isolate of nosocomial infection), E.coli(isolates of community acquired infection), C. albicans(isolates of community acquired infection), K. pneumonia (isolates of community*

			acquired infection)
12	*Syzygium aromaticum (Important constituents: eugenol, β-caryophyllene, eugenyl acetate)*	*S. mutans ATCC-700610, S. aureus ATCC-29213, E. faecalis ATCC-29212, S. bovis ATCC 9809, P. aeruginosa ATCC-27853, S. Typhimurium ATCC-13311, E. coli ATCC-25922, C. albicans ATCC-10231, K. pneumonia ATCC-700603, E. coli(isolate of nosocomial infection), E. coli(isolates of community acquired infection), C. albicans(isolates of community acquired infection), K. pneumonia (isolates of community acquired infection).*	*E. coli(isolates of community acquired infection)*
13	*Cinnamomum zeylanicum (Important constituents: eugenol, linalool, piperitone)*	*S. mutans ATCC-700610, S. aureus ATCC-29213, E. faecalis ATCC-29212, S. bovis ATCC 9809, P.aeruginosa ATCC-27853, S. Typhimurium ATCC-13311, E. coliATCC-25922, C. albicansATCC-10231, K. pneumoniae ATCC-700603, E. coli(isolate of nosocomial infection), E. coli(isolates of community acquired infection), C. albicans(isolates of community acquired infection), K. pneumoniae(community acquired infection)*	*E. coli(isolates of community acquired infection)*
14	*Unripe banana (Musa sapientum) (Important constituents: Polyphenols, Phytosterols, starch, fructants)*		*E. coli ATCC 25922, E. coli, P. aeruginosa and Shigella flexneri*
15	*Turmeric (Curcuma longa)*		*E. coli ATCC-25922, E. coli,P. aeruginosa, S. flexneri*

	(Important constituents: phenols andterpenoids)		

1.9. Herbal Drug Resistant Mechanisms

Herbal drugs are often reported to be important alternatives for MDR strains, and it is shown that bacteria can not develop resistance to herbal medicines. It has been reported that some bacteria has natural resistance to some of the herbal medicines but there is no clear understanding about the resistance mechanisms of microorganisms against these naturally occurring antimicrobial compounds.

CDC reported that alternative therapies and herbal drugs are also not the final shot to treat infections and patients with chronic diseases commonly follow herbal drugs for cure . Bacteria, in general, have the genetic ability to transmit and acquire resistance to therapeutic drugs used against them. Even in the food processing industries where herbal compounds are used since a long time, the most urgent problem is that there is still little understanding of the effectiveness of the use of classical preservatives and naturally occurring antimicrobial biomolecules (biological, ‘‘natural’’ preservatives) in conjunction with other common components of food preservation systems.

The genetic approach to reveal the herbal drug resistant genes are yet to be studied however, deletion of rpoS gene in *E. coli* was associated with decreased resistance to carvacrol similarly, deletion of the sigB gene in *Listeria monocytogenes* reduced the resistance to carvacrol. However rpoS gene deals with survival of organisms under stress conditions which may or may not be directly involved in resistance to carvacrol. Many scientific reports are available regarding the application of herbal extracts for antimicrobial, therapeutic and other therapeutic purpose in human and animals. However, literature is scant on the herbal antimicrobial drug resistance (HADR) and still less on mechanism of HADR.

1.10. Quality Control of Herbal Drugs and WHO Recommendations

Many countries with rich biodiversity like Africa, China, and India practice herbal and traditional medicine since time immemorial. Among European countries, Germany alone reported that more than 70 % of its population uses natural products. The herbal medicines as such have great potential in prevention and therapeutics and also have huge demand as herbal-derived remedies. So, it is need of the hour for powerful and deep assessment of pharmacological qualities and safety of herbal drugs.

The safety in use of herbal medicine is a very important public health aspect as the population using these medicines around the globe is enormous. The poor quality and lack of knowledge on usage of herbal drugs can be associated with adverse effects such as increased prothrombin times, severe kidney failure, fatal case of interstitial pneumonia, intracranial hemorrhages etc. Herbal drugs more commonly found to be effective in combination with synthetic drugs to treat the MDR pathogens in vitro [39] however, the adverse reactions due to combination of these drugs in vivo are yet to be analyzed for final use. Many WHO member countries are bound to regulate herbal medicines. Though the WHO, 2004 discussed and documented about the safety monitoring of the herbal medicines with respect to adverse reactions but could not analyze the development of herbal drug resistance in pathogens. Therefore, it is must to decide the amount of herbal product(s) to be used for determining herbal drug sensitivity similar to the standards available for antimicrobial drugs. In lack of standards, confusing literature will keep on emerging giving false impression of affectivity of herbal drugs on microbes. Besides, the lot of data generated in different labs using different standards poses difficulty in meta-analysis of the information to draw a useful conclusion for future clinical use of herbal antimicrobials.

2. LITERATURE REVIEW

Nair et al. (2012)[17] investigated the antioxidant activity in five species of *Rauvolfia viz., R. serpentina, R. beddomei, R. micrantha, R. densiflora and R. tetraphylla* collected from the southern Western Ghats of Kerala. The study revealed that *R. serpentina* exhibited the highest total phenolic content (44.91±2.28 mg GAE/g) while, *R. tetraphylla* had the highest flavonoid content (23.43± 1.73 mg/g quercetin equivalents) among the five species. *R. serpentina* showed DPPH radical scavenging activity (93.1 ± 0.06%). *R. serpentina* contained tocopherols (25.41± 1.08 mg/g dry weight) with the highest levels of all the isoforms, α, β, γ, and δ tocopherols. R. beddomei revealed the lowest content of vitamin E. Ascorbic acid was the most abundant vitamin in all the studied *Rauvolfia* species. *R. densiflora* gave highest levels of ascorbic acid (77.82 ± 3.97 mg/g dry weight) and metal chelating activity among the five species. *R. tetraphylla* revealed the highest concentration of β-carotene (73.61±3.85 mg/g dry weight). Lycopene was found in very low amounts while comparing with other nutrient compositions and the maximum amount was in *R. tetraphylla* (0.09±0.002 mg/g dry weight) and least amount was in R. beddomei (0.01± 0.001 mg/g dry weight). The results of this study provided an alternative way of utilizing *Rauvolfia* leaf as a readily accessible source of natural antioxidant in food, cosmetic and pharmaceutical industry.

Chatterjee et al. (2005)[18] isolated 18-hydroxy-yohimbine, ajmaline and spirobenzyl isoquiniline group of alkaloids from *R. densiflora*. Apart from being used in the treatment of hypertension, popularly it finds usage in the traditional system of medicine in the treatment of rheumatism, maternity complications, beri beri, syphilis, dysentery, diabetes, asthma, snake bite, skin diseases and gastrointestinal problems. The Indian medicinal plant, *R. serpentina* was analyzed for the chemical composition, vitamins and minerals. The results revealed the presence of bioactive constituents, comprising alkaloids, saponins, flavonoids, phenols and tannins. It is a source of many pharmacological and medicinally important phytochemicals such as reserpine, ajmaline, densiflorine, rescinnamine, isoreserpinine, reserpiline, reserpinine and sarpagine densiflora is an economically important medicinal plant, due to the indole alkaloids in its part. So far, work has been done to bridge up the vast ethnomedicinal utilization of this plant and its active principles related to the treatment of various

ailments. The present study is an attempt to explore the phytochemicals and bioactivity of *R. densiflora*.

Shunmugapriya et al., (2012)[19] Phytol was detected in the whole plant of *R. densiflora* which was also found to be effective at different stages of arthritis. It was found to give good as well as preventive and therapeutic results against arthritis. The result showed that the reactive oxygen species-promoting substances such as phytol, constituted a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases.

Srinivas et al. (2000)[20] showed that a dose of 50 mg/kg of *L. aspera* dried leaf powder in 2% gum acacia showed significant anti-inflammatory activity, which was found to be better than acetylsalicylic acid in the carrageenin-induced paw edema model and less active than phenylbutazone, when tested in cotton pellet-induced granuloma in rat model.

Saha et al. (2007)[21] reported that the semisolid mass from the yellow-colored band obtained from the methanol extract of *L. lavandulaefolia* showed significant dose-dependant anti-tussive activity reported that the ethanol extract of the aerial part of *L. lavandulaefolia* significantly reduced the incidence and severity of diarrhoea in the castor-induced diarrhoea in rats. The methanol extracts of whole plant of *L. lavandulaefolia* possess a dose-related strong hypoglycemic activity and have similar potency to that of glibenclamide at an oral dose of 400 mg/kg.

Mukundray *et al. (2007)*[22] reported that menthone, pulegone, and piperitone-rich essential oils of *L. glabrata* possessed significant antimicrobial activity against selected Gram- positive and negative bacteria and fungal strains at a concentration of 0.45 to 1.14 mg/ml. , reported that the alkaloid fraction of the methanol extracts of L. aspera flowers exhibited the antimicrobial activity. The methanol extract of L. zeylanica and 80% ethanolic extract of *L. aspera* leaves were found to exhibit potent inhibitory activity against Staphylococcus aureus and Bacillus

Ara , (2009)[23] However, the uncontrolled production of oxygen derived free radicals is associated with the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis, arteriosclerosis and degenerative processes associated with ageing. Exogenous chemical and endogenous metabolic processes in food system might

produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Antioxidants are considered to be the first line of defense against free radical damage. Antioxidants are radical scavengers, which protect the human body against free radicals.

Kumar , (2009)[24] An antioxidant is a molecule that decreases or inhibits the oxidation of other substances as a trapper of free radicals. Free radicals of reactive oxygen species are formed in our body as a result of biological oxidation. The over production of free radicals cause damage to the body and contribute to oxidative stress. Scavenging of reactive oxygen species (ROS) is important in preventing potential damage to cellular components such as DNA, proteins and lipids. Scientific evidence suggests that antioxidants reduce the risk of chronic diseases, including cancer and heart disease. The human body system has a number of mechanisms to eliminate the free radicals formed. When the normal levels of antioxidant defense mechanism is not sufficient for the eradication of free radical induced injury, the administration of antioxidants will have a protective role to play.

Bhoj Singh et al., (2017)[25] explained Emergence of drug resistant microbes and their global spread is the biggest public health dilemma of the day. Enteropathogens are the biggest killers of neonates all over the globe. This study was conducted to understand antimicrobial drug resistance in bacteria causing enteric infections. A total of 199 bacterial strains isolated from faecal samples of diarrhoeic buffalo calves, foals, children , goat kids, piglets, chicks, pups and cattle calves, belonging to 21 genera of enteropathogens were tested for their sensitivity to 8 herbal antimicrobials and 25 conventional antimicrobials. Of the tested strains 38.2%, 29.6%, and 12.1% strains were resistant to extended spectrum β-lactam drugs, carbapenems, and produced metallo-β-lactamases (MBL), respectively. Of the 24 strains positive for MBL, 22 were New Delhi metallo-β-lactamases (NDM) producers and two produced Verona integron encoded MBL (VIM). Both the VIM positive strains were Shewanella species and 6, 7, and 9 NDM producers strains belonged to *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*, respectively. About 60% strains had multiple drug resistance (MDR) and 7.5% had multiple herbal antimicrobial resistant (MHAR). Among the herbal antimicrobials ajowan essential oil (AEO) was the most effective and inhibited all the strains, followed by

cinnamaldehyde (98.2%), cinnamon essential oil (96%), holy basil essential oil (92.5%), carvacrol (91.9%), thyme essential oil (87.1%), Zanthoxylum rhetsa essential oil (21.3%) and patchouli essential oil (6.6%). Tigecycline was the most effective (in vitro) antibiotic on the strains tested inhibiting 83.7% strains followed by chloramphenicol (81.2%), moxalactam (81.1%), imipenem (78.2%), gentamicin (74%), and colistin (71.4%), other drugs could inhibit less than 70% of the strains. Erythromycin (1.3%) and ampicillin (17.1%) were the least effective antibiotics. Study revealed high levels of antimicrobial drug resistance in enteropathogens with a ray of hope with herbal antimicrobials.

Harish, et al.,(2017)[26] illustrated Indiscriminate and irrational use of antibiotics has created an unprecedented challenge for human civilization due to microbe's development of antimicrobial resistance. It is difficult to treat bacterial infection due to bacteria's ability to develop resistance against antimicrobial agents. Antimicrobial agents are categorized according to their mechanism of action, i.e., interference with cell wall synthesis, DNA and RNA synthesis, lysis of the bacterial membrane, inhibition of protein synthesis, inhibition of metabolic pathways, etc. Bacteria may become resistant by antibiotic inactivation, target modification, efflux pump and plasmidic efflux. Currently, the clinically available treatment is not effective against the antibiotic resistance developed by some bacterial species. However, plant-based antimicrobials have immense potential to combat bacterial, fungal, protozoal and viral diseases without any known side effects. Such plant metabolites include quinines, alkaloids, lectins, polypeptides, flavones, flavonoids, flavonols, coumarin, terpenoids, essential oils and tannins. The present review focuses on antibiotic resistance, the resistance mechanism in bacteria against antibiotics and the role of plant-active secondary metabolites against microorganisms, which might be useful as an alternative and effective strategy to break the resistance among microbes.

Bahorun et al. (2005)[27] studied the phytochemical constituents of *Cassia fistula.* This plant is an important source of naturally occurring bioactive compounds. Polyphenolic abundantly present in both in vivo and in vitro extracts may prove to be very important, non toxic chemopreventive agents against various oxidative stress. *Cassia fistula* callus culture could supply potent oxidative and chemoprotective components like flavonoids and anthrquinones. It has become important to evaluate

how the bioactive components in the plant extracts effect cellular signaling process and modulate oxidative stress mediated response. Integrating traditional medicine in to health system require both demonstration of clinical and biochemical evidence of efficacy

Nino et al. (2006)[28] Studied the antibacterial, antifungal, and cytotoxic activities of eleven Solanaceae plants from Colombian biodiversity. The hexane, dichloromethane and methanol extracts of 11 Solanaceae plants collected in Regional Natural Park, Ukumari (RNPU) Colombia were evaluated for their antibacterial, antifungal activities through agar well diffusion method and for cytotoxic activity by brine shrimp lethality assay. The bacterial strains include *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* For antimycotic activity, tests were performed with *Candida albicans, Aspergillus fumigatus* and *Fusarium solani.*

Agyare, et al., (2006)[29]studied the antimicrobial activity and phytochemical analysis of some medicinal plants from Ghana. The methanol and petroleum ether extracts of leaf and stem bark of *Nauclea latifolia, Bridelia atroviridis* and *Zanthoxylum gilletii* showed antimicrobial activity against test organisms *(Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans* and *Aspergillus niger*).

Rahman, et al., (2007)[30] studied the antimicrobial activity of crude extract obtained from root of *Plumbago zeylanica*. Ethanolic extract investigated for 11 human pathogenic bacteria and six phytopathogenic fungi. Among the bacteria *Vibrio cholerae* was found to be most sensitive showing highest diameter of zone of inhibition and lowest minimum inhibitory concentration (MIC) value (200 µg/ml).

Pawar, et al., (2009)[31] were evaluated central nervous system activity of different leaf extracts of *Lagenaria siceraria.* In their study, three extracts of leaves, petroleum ether, chloroform and methanol were used to study the CNS depressant activity in several animal models. In phytochemical screening the flavonoids, steroid, alkaloid, tannin, and saponin were detecte.

Modgil et al., (2004)[32] were determined carbohydrate and mineral content of Chyton (Sechium edule) and *Lagenaria siceraria*. Both plants were analyzed for their carbohydrate content viz, crude fiber, reducing sugar, non-reducing sugar and

different dietary fiber constituents like NDE, ADF, legine, Cellulose and hemicelluloses and minerals.

Deore et al., (2009)[33] were studied in vitro antioxidant activity and quantitative estimation of phenolic content of *Lagenaria siceraria*. Phytochemical screening of the crude ethanolic extract of fruit revealed the presence of flavonoids, saponins, glycosides and phenolic compounds which bears antioxidant activity.

Clautilde Teugwa et al. (2013)[34] extracted storage proteins of five species of *Cucurbitaceae*, including *Lagenaria siceraria*. The different families of storage proteins were following differential solubility. The analysis of these proteins was done by electrophoresis in non-denaturing and denaturing conditions. The results showed that among the proteins extracted, globulins constitute the most abundant class of storage proteins in all five species selected. The results of electrophoresis showed that all species possess acidic and neutrals albumins and globulins, with molecular weight of protein subunits ranging from 6.36-44.11 kDa for albumins, 6.5-173.86 kDa for globulins and 6.5-49.66 kDa for glutelins.

Chinedu, et al. (2013)[35] isolated quercetin from ethyl acetate fraction of *L. siceraria* fruits which was confirmed by Co-TLC, UV and FTIR analyses. In addition, a simple, rapid, precise and accurate high-performance thin-layer chromatographic (HPTLC) method was also established, validated for flavonoid quercetin. The linearity of the method was investigated in the range of 1-3.5 ng mL1 . Percentage recoveries for quercetin were found to be 99.79%.

Rahul Mohan et al. (2012)[36] isolated a new phenolic glycoside (E)-4-hydroxymethyl-phenyl-6-O-caffeoyl-β-D-glucopyranoside and identified together with 1-(2-hydroxy-4-hydroxymethyl)-phenyl-6-O-caffeoyl-β-D-gluco-pyranoside, protocatechuic acid, gallic acid, caffeic acid and 3,4-dimethoxy cinnamic acid. The antioxidant ability of (E)-4-hydroxymethyl-phenyl-6-O-caffeoyl-β-Dglucopyranoside was studied against 3-(4,5-dimethylthiazole-2-yl)-2,5- diphenyltetrazolium bromide (MTT) reduction assay, superoxide scavenging activity, reducing power assay and linoleic acid peroxidation assay. The compounds were found to possess promising antioxidant capacity.

3.PLANT PROFILE

3.1 Taxonomy of *Enicostemma littorale*

Kingdom: *Plantae*

Subdivision: *Angiospermae*

Class: *Dicotyledonae*

Subclass: *Gamapetalae*

Serius: *Bicarpellatae*

Order: *Gentianales*

Family: *Gentianacae*

Genus: *Enicostemma*

Species: *Littorale*

Table No: 4: Indian Chhota Chirayta (*Enicostemma littorale*)

Latin Name	*Enicostemma littorale*, *E. hyssopifolium*, *E. axillare*
Sanskrit Name	Nagajihva, Mamajjaka, Nahi, Tiksnapatra, Vitikshnika, Krimihrit
English Name	Indian Gentian
Common Name	Chhotaa Chirayataa, Naai, Vellargu, Mamejavi, Nelaguli

Table No: 5: Various Properties of species *Enicostemma littorale*

Ayurvedic Properties and Action:	
Rasa	Tikta
Guna	Laghu, Ruksha
Virya	Ushna
Vipaka	Katu
Karma	Dipana, Raktasodhaka, Amapachana, Sarala, Yakriduttejaka, Soatra, Pramehaghna, Vishaghna[1].

3.1.1 Phytochemistry

Whole plant gave alkaloids-gentianine, erythrocentaurin, enicoflavine and gentiocrucine; flavonoids-apigenin, genkwanin iso-vitaxin, swertisin, saponarin and 5-O-glucoside derivatives of sylwertisin and isoswertisin; glucosides-swertiamarin, a triterpene betulin. Swertisiode exhibited hypotensive activity.

3.1.2 Pharmacological Actions

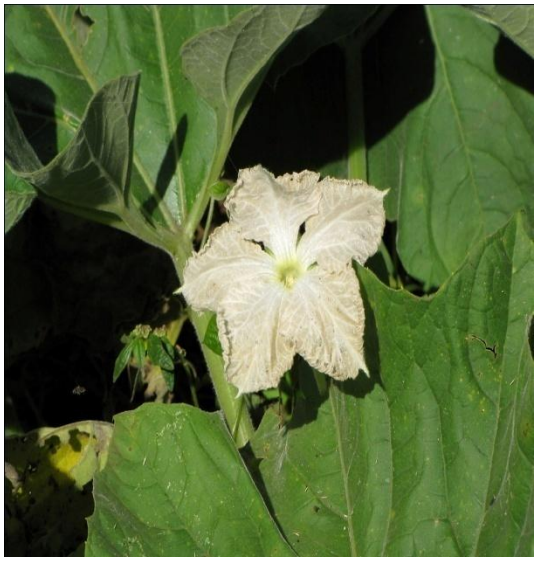
It is digestive, carminative, stomachic, laxative, depurative, antiinflammatory, liver tonic, antidiabetic.

3.1.3 Medicinal Use

It is useful in diabetes, flatulence, colic, constipation, glycosuria and intermittent fever

Lagenaria siceraria L. siceraria commonly known as Bottle gourd is official in Ayurvedic Pharmacopoeia. It is one of the excellent fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health.

3.2 Taxonomical of *Lagebaria siceraria*



Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Cucurbitales*

Family: *Cucurbitaceae*

Genus: *Lagenaria*

Species: *L. siceraria*

Part used: Fruit, root, leaves and seed oil.

2.2.1. Common vernacular names

Sanskrit : Alabu, Tumbi Ishavaaku, Katutumbi, Tiktaalaabu, Alabu.

Bengali : Laus, Lokitumbi

English : Bottle Gourd

Gujrati : Dudi, Tumbadi

Hindi : Lauki, Ghia

Kannad : Isugumbala, Tumbi

Malayalam : Chorakka, Churan, Choraikka, Piccura, Tumburini, Cura, Tumburu

Marathi : Phopla

Punjabi : Tumbi, Dani

Tamil : Shorakkai, Surai, Suraikkai

Telgu : Sorrakaya, Anapakaya

Urdu : Ghiya, Lauki

3.2.2. Uses

L. siceraria fruits are known to have diverse pharmacological benefits and were used traditionally for the benefits as an antidote to a number of snakes poison, as diuretic, aphrodisiac, tonic and in common fever. The syrup made from the fruit is used in the management of bronchial anarchies like cough & asthma. Bottle gourd is variously referred as sorakaya, anapakaya, anamgapkaya, burrakaya, a in the vernacular language by the tribal communities. Several utensils like spoon, bowls, and bottles for domestic purpose are made of dried shells of the fruit. Dried shells of the fruit are used to carry liquids in tribal areas of Khammam district. Musical instruments and pipes are being made from the dried fruits and are also used to float on the water surface in water bodies. The amount of calories in the bottle gourd is very less hence the fruit part is used for culinary use as vegetables and pickles. It is believed that bitter principle cucurbitacin is mainly accountable for its purgative properties and hence the fruit is also used as purgative in many regions in the India. The Gutti Koya tribals exercise the utilization of bottle gourd to manage headache (external application) by preparing a mixture of seed oil of *L.siceraria* and castor oil. The pulp of the fruit is considered cool and diuretic[37].

4. AIM AND OBJECTIVE

The large numbers of bacteria are resistant to antibiotics, it will be more difficult and more expensive to treat human bacterial infections. When antibiotics fail to work, consequences include extra visits to the doctor, hospitalization or extended hospital stays, a need for more expensive antibiotics to replace the older ineffective ones, lost workdays and, sometimes, death. Antibiotic resistance is found all over the world and has become a very serious problem in the treatment of disease. While the real magnitude of the problem is unknown, the monetary cost of treating antibiotic resistant infections worldwide is estimated to be many billions of dollars per year. Some experts predict that, as resistance to antibiotics is increasing at a faster pace than it can be controlled, the future will resemble the pre-antibiotic era. Others are more optimistic that research and careful drug management can reverse the trend if global efforts are focused on recognizing and controlling it. Herbal medicine remains largely an unproven, inexact science. Although the history of herbal medicine provides decades, sometimes centuries, of anecdotal information, scientific study of herbal medicine is relatively new. Compared to the Federal Food and Drug Administration (FDA), which was founded over 100 years ago, NCCAM has only begun to scratch the surface of scientific research.

Despite the criticism of herbal medicine among mainstream medical professionals, it is wise to remember that many common drugs were derived from plant-based sources and also used against infections. For example, scientists originally derived aspirin from willow bark; herbalists prescribe white willow for headaches and pain control. Digitalis, a drug prescribed for certain heart conditions, comes from an extract of potentially toxic foxglove flowers. These are just a few examples of why it's important to consider the advantages and disadvantages of herbal treatments. In the present investigation an attempt was made to test the antimicrobial activity of *Enicostemma littorale* and*Lagenaria siceraria* by using various *in vitro* and *in vivo* methods.

1.Minimum Inhibitory Concentration Determination

2.CupPlate Method.

5. PLAN OF WORK

- Collection of the plants *Enicostemma littorale* and*Lagenaria siceraria.*
- Extraction of the selected plants
- Selection of test organisms
- Selection of solvent for extraction
- MIC determination procedure for ethanol extract
- Preparation of nutrient agar medium
- Spread plate method

6. METERIAL AND METHODS

6.1. Selection of test organisms

The test organisms selected for the study are representatives of the pathogens and bacteria, which are recommended by various pharmacopoeias for antibiotic assays.

6.2. Preparation of the plant extract

The leaves were dried to a constant weight at 18°C in an enclosed air conditioned research laboratory. The dried leaves were blended to powder to increase the surface area for extraction and divided into two equal parts for both soxhlet and cold extraction procedures. The entire weight of the leaves powder was 100g.

6.3. Phytochemical test

Chemical tests performed in the screening and identification of phytocemical constituents in the tested medicinal plants were carried out in extracts as well as powder specimens using the standard procedures.

6.3.1.Maeyer's test

0.355 g of mercuric chloride was dissolved in 60 ml of distilled water.5.0g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

6.3.2.Dragendroff's reagent

Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water.

Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A&B) were mixed in 1:1 ratio.

6.3.4.Test for alkaloids

About 0.5 to 0.6 g of the aqueous alcoholic plant extract was mixed in 8 ml of 1% HCL,warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendroff's).

6.3.5. Test for steroids

About 0.5 g of aqueous alcoholic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid.

6.3.4. Test for terpenoids

An aliquot 0.5 ml of aqueous alcoholic extract was mixed with 2 ml of CHCI3 in a test tube. 3 ml of concentrated H2SO4 was carefully added to form a layer.

6.3.5. Test for flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acidwere added and boiled for five minutes.

6.3.6. Test for tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filteration method used here was the normal.

6.3.7. Test for phytosterol

The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling ,cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube.

1. Foam test : 5 ml of the test solution taken in a test tube was shaken well for five minutes.
2. Olive oil test : Added a few drops of olive oil to 2 ml of the test solution and shaken well.

6.3.8. Test for glycosides

1.Keller-Killani test : Added 0.4 ml of glacial acetic acid and a few drops of 5% ferricchloride solution to a little of dry extract.Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully.

2. Hydroxyanthraquinone Test : To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution.

6.3. MIC determination procedure for ethanolic & aqueous extract

MIC of the extracts were determined by diluting the various concentrations (0.0-36, 0.0-41, and 0.0-37 mcg/ml) of A,B, and C respectively. Equal volume of the extracts and nutrient broth were mixed in the test tube. Specifically 0.1ml of standardized inoculums of 1 to 2 X 107 cfu/ml was added to each tube. The tubes were incubated aerobically at 37o C for 18-24hrs. Two control tubes were maintained for each test batch. This is as follows: tube containing extracts and the growth medium without inoculums (antibiotic control) and the tube containing the growth medium, physiological saline and the inoculums (organism control). MIC was determined as the lowest concentration of the extracts permitting no visible growth (no turbidity) when compared with the control tubes. The MBC was determined by subculturing the test dilution on fresh solid medium and further incubated at 37^{o} C for 18-24hrs. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC.

6.4. Preparation of nutrient agar medium

Readymade dehydrated medium supplied by Hi Media was used for testing the anti microbial activity of plant extracts. The dehydrated medium was dissolved in 100ml of distilled water and heated to boiling to dissolve the medium completely following the instructions given by manufacturer. The medium was distributed on clean glass tubes and plugged with cotton and sterilized by autoclaving at 15lb/sq.inch pressure at 121ºC for 20 minutes.

6.5. Spread plate method

20ml of nutrient agar media was transferred into each petri plate. The petri plates were left undisturbed for 1-2hrs. 100 µl of each pure culture was transferred

into petri plates using micro pipette. The pure cultures were evenly spread with the help of sterile bent glass rod. They were kept for incubation for 24hrs.

6.6. Procedure for Antibacterial Activity Testing

Drug substances that either suppress or influence the growth of microorganisms are generally analyzed by microbial method. The procedure employed for this testing is Cup plate method or Agar well diffusion method.

6.6.1. Cup plate method

The antibacterial activity of the extracts was determined by using the agar well diffusion technique. Mueller- Hinton agar plates (Himedia, Mumbai) were seeded with 0.1 ml of overnight culture, allowed to incubate for 24hrs. Cups were made in Petri plates using sterile cork borer (0.85 cm) and 50µl of each extract was added into each well. Then bacterial plates were incubated at 37˚ C 24 hrs[6]. Each test compound has got six bores for which zone of inhibition diameter and mean values were determined. Antibacterial activity was determined by measurement of zone of inhibition around each well in plate using zone reader[7]. Measured inhibition zones were recorded as mean diameter in mm[8]. Gentamycin antibiotic was used as control[38-40].

6.6.2. *In vivo* anti bacterial activity

Mice were divided into 6groups and each group consists of six animals. Groups were divided shown0 below:

1. Control group Salmonella-infected (SI)
2. Positive control Salmonella-infected + Gentamycin.
3. Treatment group- 1: Aqueous ethanolic extract of *Lagenaria siceraria* 200mg/kg
4. Treatment group-2: Aqueous ethanolic extract of *Lagenaria siceraria* 400mg/kg
5. Treatment group-3: Aqueous ethanolic extract of *Enicostemma littorale* 200mg/kg
6. Treatment group-4:Aqueous ethanolic extract of *Enicostemma littorale* 400mg/kg.

All through the experiment, mice were provided with water that contained streptomycin (5 mg mL^{-1}) in order to reduce the level of facultative anaerobic bacteria that normally colonize the mouse intestine. The inhibition of the growth of test organisms in mice was then determined by monitoring *S. typhimurium* in the feces of

the mice. Briefly, *S. typhimurium* (JOL 389) was grown overnight in Luria–Bertani broth (Difco), centrifuged, washed in phosphate-buffered saline (PBS) and then diluted into 20% sucrose to achieve a final concentration of 1×10^5 CFU. The SI and SIPG groups exclusively were then inoculated using gavage needle orally with approximately 10^5 CFU of *S. typhimurium* in a 0.1 mL volume. One hour after infection, animals in the SIPG group were orally administered 5 mg (using gavage needle) of the PGPE daily, whereas CON and SI animals were not. Fecal samples were then collected 0, 1, 2, 3, 4 and 5 days after the bacterial suspensions were administered and the numbers of the bacteria per gram of feces were determined..

7.RESULT AND DISCUSSION

Hydroalcholic extract of *Lagenaria siceraria* at the dose range from 1000 µg/ml to 32.5 µg/ml by using two fold serial dilution technique against various bacterial strains was studied. The results revealed that *Lagenaria siceraria* showed a minimum inhibitory concentration (MIC) at 250 µg/ml to 500 µg/ml of the broth against all bacterial strains. The results were comparable with positive control which was showed MIC at 1.8625 µg/ml to 3.625 µg/ml. The similar findings were also observed in zone of inhibition by using cup plate method (Table No.2 & 3). Since the drug showed better activity against various bacterias, it has given us a lead to select *S. typi* for our further anti bacterial study.

The results of *Enicostemma littorale* at the dose series from 1000 µg/ml to 32.5 µg/ml by using two fold serial dilution method against a range of bacterial strains were studied. The results revealed that the *Enicostemma littorale* showed a minimum inhibitory concentration (MIC) at 250 µg/ml to 500 µg/ml of the broth against all bacterial strains. The results were comparable with positive control which was showed MIC at 1.8625 µg/ml to 3.625 µg/ml. The similar findings were also observed in zone of inhibition by using cup plate method (Table No. 3). Since the drug showed better activity against various bacterias, it has given us a lead to select *S. typi* for our further anti bacterial revise. **Phytochemical Analysis**

The phytochemical screening results revealed that the after which it was observed whether the alkaloids were present by the indication of turbidity and/or precipitate formation. The colour changed from violet to blue or green in some samples indicated the presence of steroids. An interface with a reddish brown coloration was not formed in the absence of terpenoids, as positive result. Red coloration identifies the presence of flavonoids (Shinado's test). A colour change was observed in the test tube, which indicated in the presence of tannins. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols. Below two observation indicated presence of Saponins Formation of stable foam confirmed the test The formation of a soluble emulsion confirmed the test.

The formation of blue colour in acetic acid layer confirmed the test. The Formation of red color confirmed the test. Above two observation indicated presence of glycosides.

Table. No: 2: Phytochemical Analysis

S.No	Phytochemicals	
1	Alkaloids	+
2	Steroids	+
3	Terpenoids	-
4	Flavonoids	+
5	Tannins	+
6	Phytosterol	+
7	Saponin	+
8	Glycosides	+

+, Presence of the compound

-, Absence of compound

Table. No: 2: Effect of *Lagenaria siceraria* on selected bactrial strains (Two fold serial dilution)

S. No	Organisms	*Lagenaria siceraria* Extracts MIC Values µg/ml	*Enicostemma littorale* Extracts MIC Values µg/ml	Standard drug MIC Values µg/ml
1.	*S.typi*	500	250	3.625
2.	*S. enteritidis*	500	250	3.625
3.	*E.coli*	500	500	3.625
4.	*S. aureus*	500	500	1.8625
5.	*K. pneumoniae*	250	500	1.8625

Standard drug – Gentamycin

Table No: 3: Effect of *Lagenaria siceraria* on selected fungal strains (Cup Plate method)

S.No	Organisms	Concentrations extract	Zone of inhibition(cm)				Standard drug Zone of inhibition values (cm)	
			Lagenaria siceraria		*Enicostemma littorale*			
			Well 1	Well 2	Well1	Well 2	Well 1	Well 2
1.	*S.typi*	1000 µg/ml	1.2	1.4	1.6	1.1	1.3	1.6
		2000 µg/ml	2.1	2.4	2.9	2.1		
2.	*S. enteritidis*	1000 µg/ml	1.3	1.4	1.7	1.2	1.2	1.7
		2000 µg/ml	2.3	2.4	3.2	2.3		
3.	*E.coli*	1000 µg/ml	1.2	1.1	1.9	1.1	1.8	1.9
		2000 µg/ml	2.7	2.1	4.0	2.6		
4.	*S. aureus*	1000 µg/ml	1.5	1	1.7	1.5	1.8	1.7
		2000 µg/ml	2.7	2.3	3.6	2.7		
5.	*K. pneumoniae*	1000 µg/ml	1.1	1.5	1.8	1.1	1.5	1.6
		2000 µg/ml	2.5	2.7	2.9	2.5		

***in vivo* anti bacterial activity**

Enicostemma littorale extract on experimental on SI mice was studied. *Enicostemma littorale a siceraria* at 400mg/kg forbidden the infection maximally on 4^{th} day of treatment. The complete abolition of infection has observed only on 5^{th} day of post infection. The results were compared with gentamycin, which has controlled the infection effectively from 3^{rd} day of treatment and eradication of infection was found maximum on 4^{th} day of treatment.

The results indicated that *Lagenaria siceraria* at 2mg /kg and 400 mg/kg dose levels were showed effective inhibitory action against bacteria on 3^{rd} day onwards in animals. *Lagenaria siceraria* at 400mg/kg controlled the infection maximally on 4^{th} day of treatment. Whereas complete eradication of infection has observed only on 5^{th} day of post infection. The results were compared with gentamycin, which has controlled the infection effectively from 3^{rd} day of treatment and eradication of infection was found maximum on 4^{th} day of treatment. The results were recorded in TableNo.&figno.to

Table. No: 4: Effect of *Lagenaria siceraria &Enicostemma littorale*against *s. typi*in mice

S. No.	Groups	Organism/ Drugs/ Salmonella	Dose mg/kg	Colony Forming Units (c.f.u.) days					
				0	**1**	**2**	**3**	**4**	**5**
1.	Control Group	CMC 0.5%	Required quantity	7.9 ± 0.08 x 10^4	9.1 ± 0.05 x 10^4	10 ± 0.3 x 10^4	13.6 ± 0.1 x 10^4	23.3 ± 0.3 x 10^4	19.5 ± 0.5 x 10^4
2.	Positive Control	Gentamycin	2	6 ± 0.06 x 10^3	7.8 ± 0.02 x 10^2	6.7 ± 0.05 x 10^2	4.1 ± 0.05 x 10^2	2.1 ± 0.03 x 10^2	Nil
3.	Treatment group- 1	AAEEL	200	3.6 ± 0.04 x 10^4	8.1 ± 0.02 x 10^3	6.1 ± 0.07 x 10^3	4.8 ± 0.03 x 10^3	3.8 ± 0.1 x 10^3	2.6 ± 0.3 x 10^3
4.	Treatment group- 2	AAEEL	400	1.6 ± 0.09 x 10^4	5 ± 0.08 x 10^3	3.4 ± 0.01 x 10^3	1.5 ± 0.03 x 10^3	5.2 ± 0.1 x 10^2	4.5 ± 0.2 x 10^2
5	Treatment group-3	AAELS	200	2.6 ± 0.04 x 10^4	7.1 ± 0.02 x 10^3	5.1 ± 0.07 x 10^3	4.8 ± 0.03 x 10^3	2.8 ± 0.1 x 10^3	2.6 ± 0.3 x 10^3
6	Treatment group-4	AAELS	400	1.6 ± 0.09 x 10^4	5.9 ± 0.08 x 10^3	4.4 ± 0.01 x 10^3	3.5 ± 0.03 x 10^3	2.2 ± 0.1 x 10^2	1.8 ± 0.2 x 10^2

Figure No: 14: Control

Figure No: 15: Positive Control

Figure No: 16: Aqueous ethanolic extract of *Lagenaria siceraria* 200mg/kg

Figure No: 17: Aqueous ethanolic extract of *Lagenaria siceraria* 400mg/kg

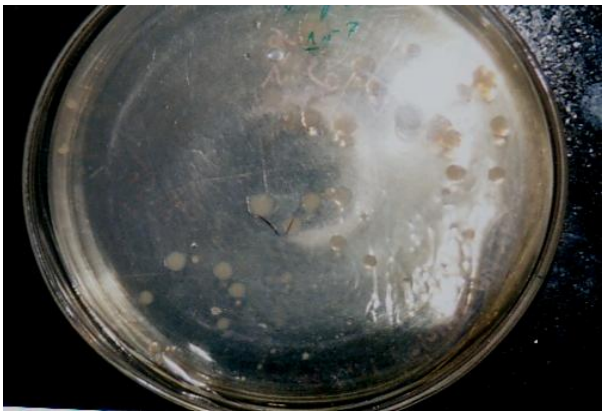


Figure No: 18: Aqueous ethanolic extract of *Enicostemma littorale* 200mg/kg

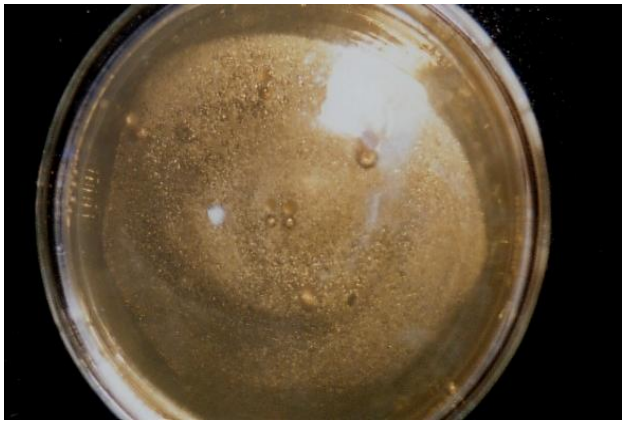
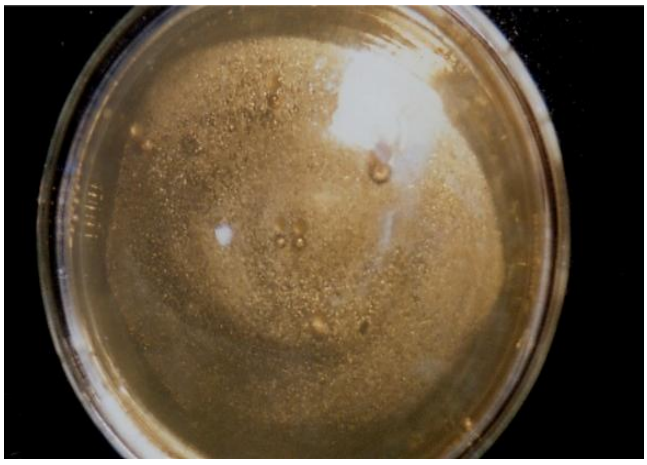


Figure No: 19: Aqueous ethanolic extract of *Enicostemma littorale* 400mg



7. CONCLUSION

In this context, the Plants are the basic source of knowledge of modern medicine. The relatively lower incidence of adverse reactions to plant preparations, compared to modern conventional pharmaceuticals, coupled with their reduced cost is encouraging both the consuming public and national health care institutions to consider plant medicines as alternative to synthetic drugs. Nowadays herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness and minimal side effect in clinical experience large numbers of plants belonging to different families have been studied for their therapeutic properties.

In this present study, the antibacterial activity of the herb *Enicostemma littorale and Lagenaria siceraria* has been planned to prove its activity against various species of microbes by spread plate method. This study has been very beneficial as it has given an evidence of utilizing this species against the particular microbes satisfactorily.

Newly, a numeral of antibiotics has lost their efficiency due to the development of resistant strains of bacteria, which has chiefly occurred through the expression of resistance genes. In accumulation to inducing resistance, antibiotics were sometimes associated with opposing effects such as hypersensitivity, immune-suppression & allergic reactions. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. This suggests that these mechanism may also provide antibacterial activity against *Salmonella* and give a reasonable clarification for the higher antibacterial activity of the EtOH extract. On the other hand, the unidentified minor components present have not been elucidated in terms of their activity. Further studies then need to be done. In the future, thorough study is needed to improved ascertain the antibacterial effect of this herb extract.

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